CUSTOMER NO. 000042131 **Docket No. 170.002**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Wei He and Wei Weng

SERIAL NO.: 10/768,350 **Group Art Unit:** 1632

FILED: January 30, 2004 **Examiner: Joanne Hama**

FOR: Genetic Modification of C57 Mice

DECLARATION OF Dr. Wei He PURSUANT TO 37 C.F.R. 1.132(a)

1. I am the co-inventor in the above referenced application. I am currently a Research Scientist at InGenious Targeting Laboratory. Inc. located at 25 E. Loop Road in Stony Brook, New York.

2. The Office Action rejected claims 3-8 under 35 USC sec 103 as obvious over Schuster-Gossler et al (2001, Biotechniques 31: 1022-1026) and Smith (2001, Annu. Rev Cell Dev Biol 17: 435-462) in view of Katayama et al. (2001, Biochemical and Biophysical Res Comm 282: 1134-1140).

3. Response in Reference to Claim 3:

When ES cells are introduced into cysts of mouse blastocysts by injection, traditionally coat color has been used to determine the percentage of the injected ES cells contributing the host blastocysts. Finding the best combination of host blastocysts/ES cells has been a major obstacle for generating genetically modified C57 mice.

Traditionally, to generate C57 knockout mice, ES cells from C57BL/6 (black) have been injected into blastocysts from Balb/c (white) and chimerism is detected by the change in coat color. Schuster-Gossler et al took ES cells from C57BL/6 and injected them into blastocysts from C57BL/6J-Tyr $^{c\text{-}2J}$ (also called C2J or albino B6) (white) and used coat color change to detect chimerism. However, in order to obtain enough blastocysts from C2J mice, a large number of C2J mice has to be used. This is a great drawback because the C2J mice are expensive and available from few vendors only. Secondly, the rate of gene transmission is not improved in Schuster-Gossler's protocol.

We were the first one to use ES cells from C57BL/6 and injected them into blastocysts of C57BL/6. We found unexpected results. The rate of gene transmission increased by about nine fold over that observed with methods described in Schuster-Gossler's paper. We have established and deposited a highly competent germline ES cell line IC1 derived from C57BL/6 strain. In the past 3 years we have carried out different experiments to develop genetically modified C57 mice, for example.

1) We injected IC1 ES cells into 124 black B6 blastocysts. 42 (34%) chimeras were obtained. Among 15 C57 knockout projects 9 projects achieved gene transmission with this method.

2) We injected IC1 ES cells into 289 albino B6 blastocysts (like Schuster). 44 (15.2%) chimeras were obtained but only one C57 knockout project achieved gene transmission among 15 C57 knockout projects.

Therefore, the method of Claim 3 of producing a transgenic mouse by injecting ES cell from C57BL/6 into blastocysts fo C57BL/6 produced a greater efficiency of germline transmission, and meets the requirement of novelty and unobviousness because of the unexpected results we obtained.

4. In reference to claim 5:

We were the first one to establish albino B6 ES cell lines from C57BL/6J-Tyr $^{c-2J}$ strain. This is the IAC1 ES cell line covered by Claim 2. When we injected albino B6 ES cells into black blastocysts we found that the rate of generating chimera was better than Schuster-Gossler's method and also better than traditional method (B6 ES cells injected into Balb/c embryos). We injected albino B6 ES cells into 41 B6 blastocysts and 12 chimeras (29%) were obtained. However, using Schuster-Gossler's method, we injected IC1 ES cells into 289 albino B6 blastocysts and only 44 (15.2%) chimeras were obtained. With the traditional method, we injected B6 ES cells into 410 Balb/c blastocysts and got 86 (21%) chimeras. Also, we found that it is easier to maintain albino B6 ES cells than commonly used 129 ES cells. As donor part, black B6 mice generate more embryos than their white counterparts and provide much better quality of embryos than Balb/c mice.

In addition, the mouse colony setup is similar to the set up commonly used 129 knockout production. Researchers still can use coat color selection to generate C57 knockout mice, they don't have to set up extra mouse colony of Balb/c or albino B6 mouse strain for blastocyst donation. Therefore, it can save money, space and animals.

In summary, the "white into black" method generated 2 times more chimeras than Schuster-Gossler's method. The number and quality of C57BL/6 embryos are about 2 times better than that of albino B6 used in Schuster-Gossler's paper and Balb/c mouse strain. Together, the "white into black" method could generate 3-4 times better results than Schuster-Gossler's and traditional methods. Also it will be more cost effective and convenient to use.

I hereby declare that all statements made herein to my knowledge are true, and all statements made on information and beliefs are believed to be true; and further, that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon.

BY: _____

Wei He, Ph.D

DATE: 8/26/05

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is transmitted by Express Class No. ED 424855190 US under 37 C.F.R. 1.10 on August 29, 2005 addressed to: Commissioner for Patents, Alexandria, VA 22313-1450.

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Signature

8/29/05